N18

N18

DATE: 9/7/56 REF: lac, gal+ gal− interacti

See EML Thesis. W1402 = W811 = lac_1^{87} gal4

W1402 Isolate from EML.

Cross-streaks on EMB lac against dlac-F'.

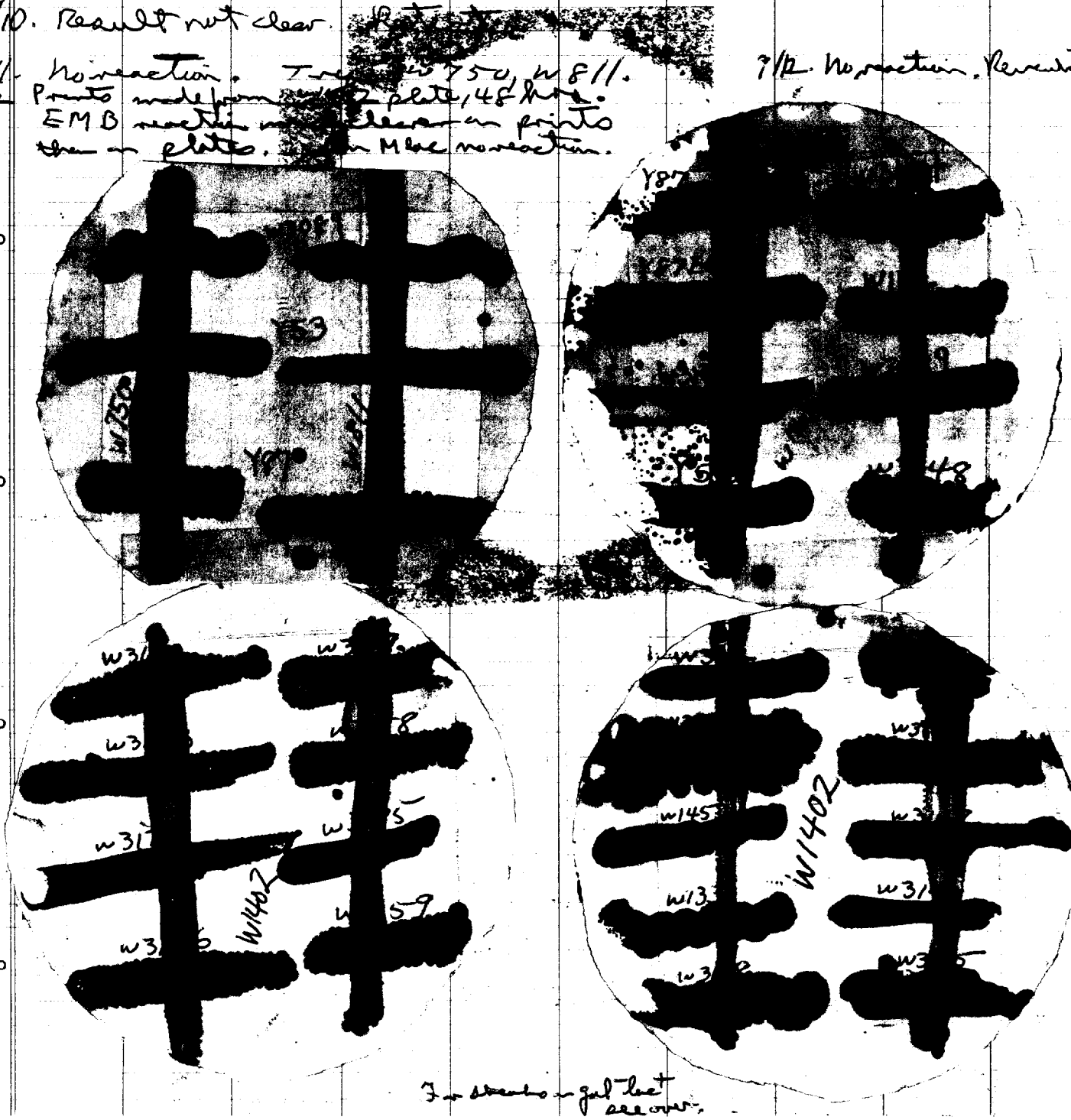
A 9/8 No interaction. Re-incubate.

9/10. Result not clear.

9/11. No reaction. T[illegible] W750, W811.

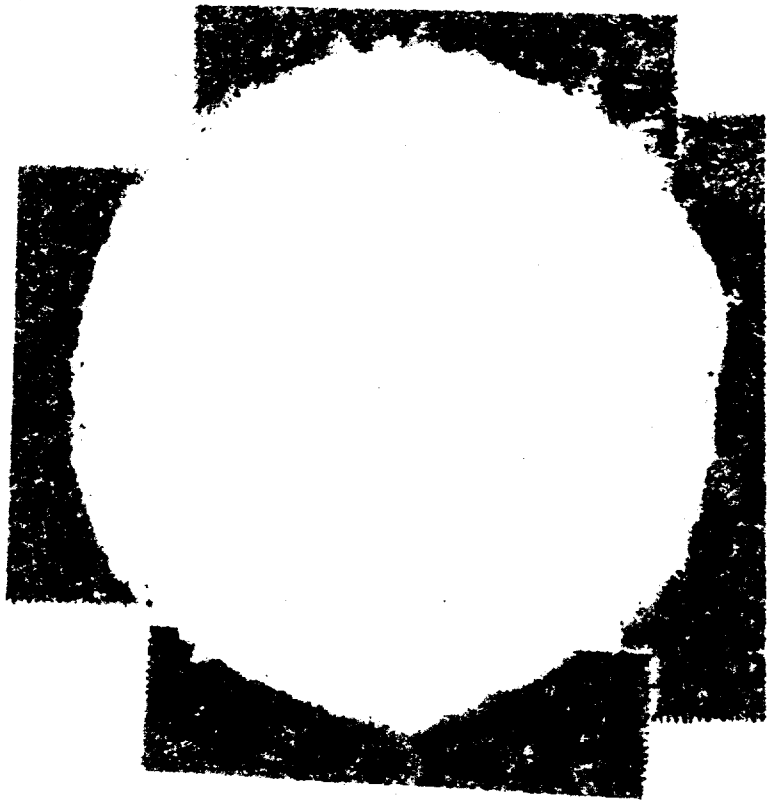
9/12 Print made from [illegible] plate, 48 hrs. EMB reaction more clear on print than on plate. [illegible] Mac no reaction.

9/12. No reaction. Re-incubate.



7+ streaks on gal lac+ see over.

10/7/56. Lac$^-$ Gal$^+$ on gal$^-$ lac$^+$ (w3091).



N19

UV-induced lac⁻ from W1895 P⁻

DATE: 9/10/56 REF:

	1	2	3	4	5	6	7	8	9	10

Single colonies 1 and 3 each spread on 8 plates B lac @ 1 drop/plate.
Exposed to Hanovia for 8 sec.

9/11 plac⁻ (?) colonies picked, streaked on B lac.

9/12. Restreaked lac⁻.

9/13. One lac⁻ from each colony (1 and 3). Restreaked on B lac.

9/14. Both colonies restreaked on B gal for stab.

9/15. N19-3 (= W3240) is gal+. N19-1 (= W3239) is gal+. 5 tubes made for sup. col.

N20

9/10/5-6

DATE: Prep. P1 lysate REF:

	1	2	3	4	5	6	7	8	9	10

9/10 11 A.M. .1 ml. of λ⁻ stocks from previous assay to 1 ml. broth in rotator. (see Hermox paper). Following λ⁻ stocks treated:

*	W2659	W3059	W3014
*	W3077	W1485	W3019
	W2964	W1655	W2915
	W3047	* W518	
	W3013	W3110	
	W3189	* W3010	
	W3136	W3017	

2 P.M. add .1 ml P1 lac / tube. Observe every hr. to 6 p.m.

7 P.M. in frig. 9/11. lysates centrifuged, decanted into vials, + CHCl3.

The ones marked * cleared completely, with debris at bottom of tube.

9/19. phage assay. Plate from 3rd dilution tube

$$\frac{I}{100} \times \frac{II}{100} \times \frac{III}{100} \times 10 = 10^{7}$$

0.1

all dil. in H_2O, begining with W1655 + T6.

last dil. in broth of W1655 + T6.

W1895 used as T1, T6 indicator

indicator cultures several days old

In P1, the indicator was the stock from which lysate was prepared.

W3014 assays 1×10^{8} P1/ml all others less.

W1655 " 4×10^{9} T6/ml

T1 lysate not good.

The "T6" lysate lyses W1366 (K6ᵀ). Other T6 preps. do not. Check the T6 preps. to locate origin of difference.

P1 cross-streaks show turbid lysis on all stocks.

mapping V_6^r lac_1^{W112} $Prol^-$ V_1^r

DATE: 9/10/56 REF:

W3236 ~~W112~~

$W1366 = F^- T^- L^- B_1^- \; lac_1^{W112} \; V_1^r V_6^r$. From stab coll. into penassay

10 ml LØ (Lennox broth) + 0.1 ml W3010. On aerator

D(0) old Pret and purify ca. 200 colonies on D(0) + Prol.

~~Wate in penassay. Centrifuged & resuspended in D(0) + prol.~~

~~Plate on B + lac.~~ $D = 4 \times 10^8 \rightarrow 50$ col/plate.

Prepare T6 and T1 phage stocks on W3010, W1655.

4 PM 1 loop W3010, W1655 in LØ, 5 tubes. 5 pm. 1 loop T1 or T6

9/11. Lysates centrifuged, decanted into vials, + several drops $CHCl_3$

9/12. Mating on ~~LØ~~ D(0) + prol.

9/13. first streak on ~~LØ~~ D(0) + prol.

9/14 second streak on B gal.

9/15 third streak on D(0) + prol. 9/16 fourth streak on D(0) + prol.

9/17. Spot on D(0) + proline for replica tests.

	1st replica	2nd replica
D(0)	D(0)	B lac
B(lac)	LØ+T6	LØ+T1
LØ+T1	Scrub block with zeocal after each use.	
LØ+T6		

N.B. W1366 was not tested as single colony before use. It is $T1^s$. The T1, T6 stocks used to score V_1, V_6 are o.K. (they are T6 from EML and T1 (W1485) from stock.

N21 A

Counts

DATE: 9/21 REF:

	1	2	3	4	5	6	7	8	9	10
	lac+, Pro+ clear; V_1 or V_6 ambiguous. Streak out.									
	plate #.			lac−	lac+ (P+)		lac+ (P−)	lac−		Total
	2			16	3		5	1		25
10	3			21	2		2	2		27
	4			18	6		4	1		29
	5			3	2		2	1		8
	6			21	4		—	4		29
20	1			16	1		4	2		23
	Total			95	18		17	11		141

~~Entire plate was plated out.~~

30		lac+ −	P− +	TL−		
	lac− P+				95	$\frac{18}{113}$ .159
	lac+ P+				18	
40	lac+ P−				17	
	lac− P−				11	$\frac{11}{28}$ = .393

~~Entire plate.~~

50				
$\frac{29}{141}$ = .206	18	95	113	$\chi^2 = \frac{7.70 \times 10^{-1}}{1.028 \times 10^{-1}}$
	11	17	28	
	29	112	141	= 7.49

V_6 Soc Pool

gal V_6 Soc Pool

gal-Soc 25.5

gal-V_6 22.7

gal-Pool 19.1

5.7
2.8
7.8
9.2
25.5

7.8
9.2
4.3
1.4
22.7

18+20=36

P V_6 Soc

Pool V_6 Soc

28+ 20

31 V_6 + Soc

27P

2.8
5.7
9.2
1.4
19.1

V_6

N21 B

DATE:

$V_6^{\sim}$ REF: V_6^{S}

Plate #	2 Loc +3 P+	2 Loc +3 P−	4 Loc −5 P+	4 Loc −5 P−	6 Loc +7 P+	6 Loc +7 P−	8 Loc −9 P+	8 Loc −9 P−	10
1									0
2									0
3									0
4									0
5			1						1
6	0	0	0	0	0	0	0	0	0
1	0	2	15	2	1	2	1	0	23 ✓
2	2 11	4 1	16 16	1 1	1 1	4 ////	0	0	25 ✓
3	1 1	1 1	20 20	2 //	1 1	1 1	1 1	0	27 ✓
4	//// 4	0	//// //// ////	1	2 //	//// 4	1	0	29 ✓
5	1 1	0	/// 3	0	1 1	2 //	0	0	✓ 7
6 (averaged twice on two plates 2 days apart)	0	0	18 //// //// ///	2 //	4	0	3 ///	2 1	29 ✓
	c.o.I	c.o.I	n.c.o.II	n.c.o.I	n.c.o.I	n.c.o.II	c.o.I	c.o.I	
	c.o.II	n.c.o.II	n.c.o.II	c.o.II	c.o.II	n.c.o.II	n.c.o.II	c.o.II	✓
	✓ 8	✓ 4	✓ 89	✓ 9	✓ 10	✓ 13	✓ 6	✓ 2	141
%	5.7	2.8	63.1	5.7	7.1	9.2	4.3	1.4	✓

DATE: REF:

I II

V_6^- Loc$^-$ P$^-$

.21 .05

	Loc$^+$	Loc$^-$
V_6^-	0	20
V_6^S	4	5

C.O. I 5/29 = .17

	Loc$^+$	Loc$^-$
P+	4	21
P$^-$	0	4

C.O. II 8/29 = .276

29/141 = .206

	P+	P$^-$
V_6^-	18	2
V_6^S	7	2

~~C.O. I+II~~ 9/29 = .310

N.C.O. I & II $\frac{18+0}{29}$ =

Doubles I & II $\frac{2+0}{29}$ = .07

P tot .21 V_6^- loc .142

~~Coincidence = 1~~ all data

C.O. I 20/141 = .142

C.O. II 29/141 = .206

Doubles 10/141 = .071

Expected = .029

V_6^- Loc$^-$ P$^-$ V_1^- [TL]

.142 .206 ? not small very small?

1366 V_6^- loc, w/12 P+ TL$^-$

w/895 P$^-$ S +

Prep. of F⁻ prototroph testers

DATE: 9/12/56 REF:

1	2	3	4	5	6	7	8	9	10

✓ 1. W3120 JL X Y10 $\xrightarrow{S\ lac}$ Second streak on S lac

stabbed as W3230.

~~2. Test N7-2 against X10 (W1895 control)~~.

~~N7-2 in assay.~~

✓ 3. Y87 X ~~W1895~~ Y10 → 9/17. ~~First~~ Second streak on B lac.

Stabbed as 23-3.

✓ 4. W145 X W1895 9/17 mating repeated in broth. ~~my W145 stock~~

my W145 is M⁻. Check stock ~~is M⁻~~ (grows in

~~Stabbed as W3236~~

cold.

5. W3140 ~~W2243~~ X W1895 9/17 mating in broth. Use

= ~~W3237~~ W2244F⁺ instead.

✓ 6. W133 X W1895 Second streak on S lac. = W3238.

~~Stabbed as W3236.~~

= ~~W3233~~ 3. ~~[illegible]~~.

Resistant Tests of T1, T6, P1

DATE: 9/20. REF:

On Blac: overnight plate.

	T1 strain	T6 (w1655)	P1 (w3014)	
w30140	S+	+	—	
w3110	S+	+	—	
w1655	S+	+	—	
w1366	R	(+)	—	Papilla from w1366 picked; necessary.
w1485	S	+	—	
	O.K.	V?	—	
w3146		+	—	remove's P1KC on the P1 cross-streaks.

New streaks made on LØ with stock T6 & P1kc.

7/23. Colony of w1485 resistant to T6? (w1655) tested against stock T6 and T6 (w1655). It is lysed by T6 (EML stock). not T6 h. Plaque

	T6	T6? (w1655)
w1485	lysis	lysis
w1485R	lysis	no lysis

New T6 lysates made from T6 (EML) on B/1, w1485. Both clear, controls very turbid. Centrifuged, chloroformed, shaken, overnight in frig.

Tests of T1, T6 show some sort of massive confusion in making stocks. T1 (B/6), T6 (B/1), and T6 (1485) are O.K. by comparison with T6 from EML and T1 (1485) from stock on B/6, B/1, w1485. w1366 is T1 S.

w1366 ~~is~~ made resistant to "T6" (w1655) appears to be T5 resistant. Unrecorded error lysate

N24
A

Stability check

DATE: 9/26/56 REF:

1	2	3	4	5	6	7	8	9	10

Plunge stocks on B loc.

P1 loc
0

P1 (w3014)
0

T1 (1485)
0

T1 (B/6)
0

T6 (1485)
0

T6 (B/1)
0

T6 (1485) EML
0

P1 (1485)
0

N25

Agar layer plate

DATE: 9/26/56 REF:

	1	2	3	4	5	6	7	8	9	10

10 A.M. 45 ml. L ø agar / plate

2 ml. L. ø top layer agar + 2 drops cells + 1 drop P1.

1 W1485 + P1 lysate

2 W1485 + (W3014) P1

3 W1366 + P1 lysate

4 W1366 + (W3014) P1

old plates 25 ml. { 5 W1485 + P1 lysate

6 W1485 + (W3014) P1 1 ml. P1 lysate (W3014).

3 pm. (W3014) P1 no perceptible lysis. Discard

P1 lysate ~~no growth complete lysis~~ on plate 3, partial lysis on plates 1 & 5. Add 4 ml L broth to plate 3. Discard.

5 pm. add 4 ml L broth to plates 1 & 5.

8 pm. decant, chloroform, store in frig. add one L broth to plates 1 and 5.

9/3/56 Test of titer.

	1485	1485 (P1)
P1 (Lennox)	++	+
P1 (first decant fr. 1485)	±	○
P1 (decanted after 24 hrs.)	±	○

N26

T6 resistance of luc-testers on B(0)

DATE: REF: T6 (B/1)

	1	2	3	4	5	6	7	8	9	10
			+ = lysis		0 = no lysis					
plate 1					plate 2					
	W3229	+	W3133	+	—		W3159	+		
	W3120	+	W3230	+	—		W3089	+		
	343	+	W3134	+	W1941	+	W3148	+		
10	W1950	+	W3157	+	W1945	+	W3152	+		
	W1951	+	W3158	+	W1948	+	W3174	+		
plate 3	W1949	+	W3156	+	plate 4 W3164	+	W3112	+		
	W3146	Resistant	W3175	Resistant	—		W2243	+		
	W1946	+	W3153	+	W3140	+	W2244	+		
20	W3237	+	—		—		W3127	+		
	W3240	+	—		—		W2245	+		
plate 5	—		W3238							
	W3128		W3239							
30	W1940		W3147							
	W1942		W3149							
	W1943		W3215							
	W1944		W3151							
	W1947		W3154							
40										
50										

check on Lac⁻ testers

N27

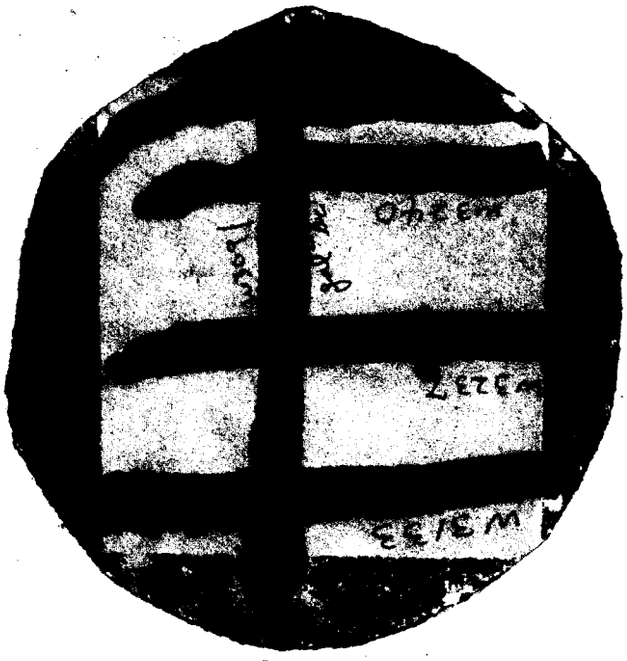
DATE: REF:

	1	2	3	4	5	6 ✓	7	8	9	10
plate 1			A			B				
		W3133	W3230	W3134	W3157	W3158	W3159			
	W3229	0	7	8	+	+	0			
	W3120	0	7	2	+	+	0			
	343	0	6	5	+	+	0			
10	W1950	12	13	3	+	+	(3)			
	W1951	20	12	3	+	+	0			
plate 2 ✓	343 test	F only	+ Hfr	F⁻	+ Hfr	~~H H H H H~~				
W3089	~~W1941~~	0	0	0	0	~~W3146~~	W3156			
W3148	20 ~~W1945~~	0	0	0	5	~~W1946~~	W3175			
W3152	~~W1948~~	0	0	0	3		W3153			
W3174	~~W1949~~	0	0	0	0		W3133			
plate 3	W3174 testers									
		Hfr F⁻	W3174	Hfr F⁻	W3174					
	30									
	W3229	0	0	0	[illegible]	W1941				
	W3120	6	[illegible]	[illegible]	+	W1945				
	~~343~~	4	0	0	0	W1948				
	W1950	+	[illegible]	0	0	W1949				
	W1951	+	[illegible]	0	+	W3146				
	40 ~~W1941~~		[illegible]	0	+	W1946				
	~~W1944~~				3174	[illegible]	W1948	1949		
					3156	0	0	0		
plate 4	W3230 test					—	[illegible]	+W3120		
	Hfr F⁻	W3230	W3230 only		W3159	0		0		
W1941	0	+ +	0		W3230	5		14		
1945	0	+ +	0			—		3140		
1948	0	+ +	0		W2244	0		0		
1949	0	+ +	0		W3127	4		1		
3146	0	+ +	0							
1946	0	+ +	0							

plate 5

Navigated colonies

all alleles ✓



27-8

27-10

27-

27-11

N27A

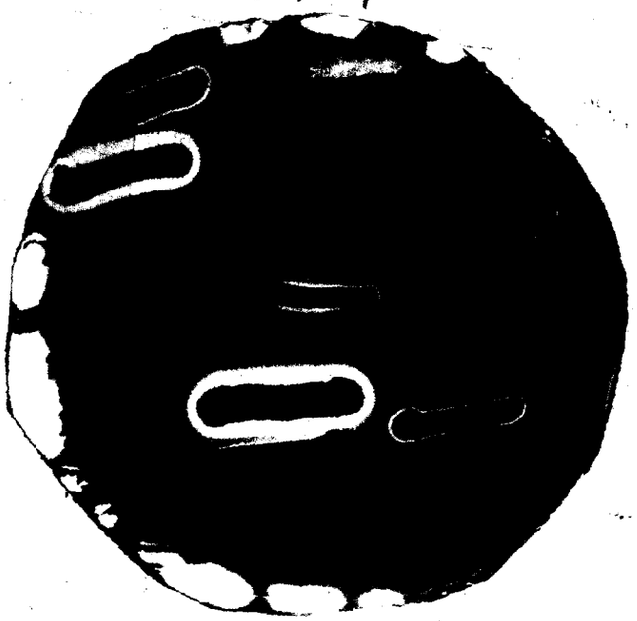
+ 24 hrs

DATE: REF:

	1	2	3	4 6A	5	6	7 6B	8	9	10
	plate 6	w3140	N7-1	w3112	W2243	W2244	w2245	w3238	w3128	w3239
		w3140	O	O	++	O	++	+	±	±
		w3229	O	O	++	O	++	+	±	±
			O	++	+-	++	++	++	±	±
					w3128+w3229					
10	plate 7 —			w3133	w3112	W2243	w2244	w2245	w3238	w3239
	O		3237	++	+	++	+	+	++	++
	O		3240	O	O	++	O	+	++	±
			w3089							
					T6 (B/1)	plate 9			plate 10	
20	plate 8	w811	T6		w811	T6		w811	T6	
	w3133	+		w3089	+		w3153	+		
	w3230	+		w3148	+		w3237	+ (0)		
	w3134	+		w3152	+		w3240	O (0)		
	w3157	+		w3174	+		w3172	+ (0)		
30	w3158	+		w3156	+		w2243	+ (0)		
	w3159	+		w3175	+	Repeated	w2244	+ (0)		
Blue	plate 11			plate 12			plate 13		B mal	
							+,- at 12 hrs.			
	w3127	+		w3147						
	w2245	—		w3149			w3133 +	w3174 +	w3127 +	
40	w3238	+		w3215			w3230 +	w3156 +	w2245 —	
	w3239	+		w3151			w3134 +	w3175 +	w3238 +	
	w3147	+		w3154			w3157 +	w3153 +	w3239 +	
	w1366	+		w3014			w3158 +	w3237 +	w3147 +	
							w3159 +	w3240	w3149 +	
50	plate 14		B mucase				w3089 —	w3112	w3215 +	
	plate 15		xylose				w3148 +	w2243 —	w3151 +	
	plate 16		MTL				w3152 +	w2244 +	w3154 +	
	plate 17		B gal							

N.B. Tomorrow test N13-2 Het against other loci.

27-17



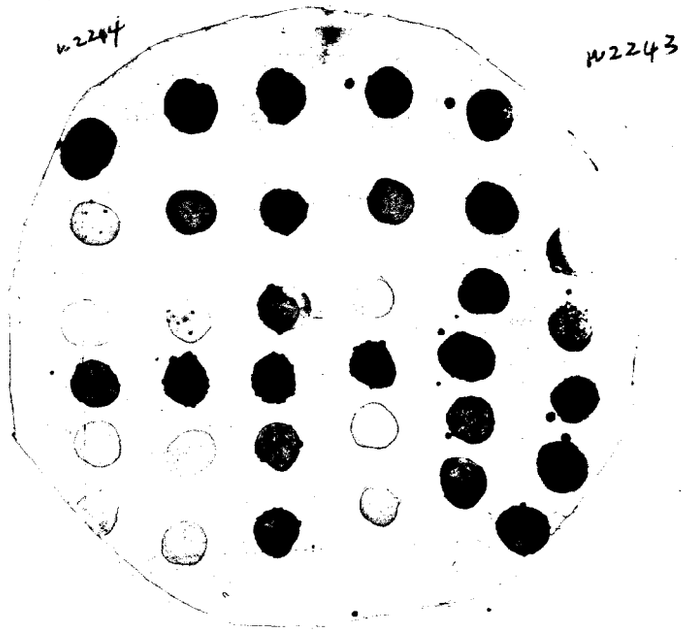
27-16

27-13

DATE: 9/27. Final readings at 12 hrs. REF:

	1	2	3	4	5	6	7	8	9	10
	plate 16 ~~B~~	BMTL ~~glucose~~	B +:- at	MTL 12 hrs.						
	w3133	+	w3174	++	w3127	+	plate 14 B glucose			
	w3230	+	w3156	+	w2245	—	at 12 hrs all negative; slowest growth			
	w3134	++	w3175	+	w3238	+	of w2243.			
10	w3157	+	w3153	+	w3239	+				
	w3158	+	w3237	wh slow	w3147	+				
	w3159	+	w3240	wh slow	w3149	+				
	w3089	+	w3112	wh slow	w3215	+				
	w3148	+	w2243	— —	w3151	+				
20	w3152	+	w2244	+	w3154	+				
	plate 15		xylose	all negative;	w2245 wh.		at 24 hrs all xylose +			
	plate 17		B gal							
	w3133	+	w3174	+	w3127	+				
30	w3230	+	w3156	+	w2245	w. slow				
	w3134	+	w3175	+	w3238	+				
	w3157	+	w3153	+	w3239	+				
	w3158	+	w3237	~~wh~~ slow	w3147	+				
	w3159	+	w3240	slow	w3149	+				
40	w3089	+	w3112	~~wh~~ slow	w3215	+				
	w3148	+	w2243	very slow	w3151	+				
	w3152	+	w2244	+	w3154	+				
	11/29/56									
50			w2243 is glucose —							
			w2245 is glucose +							

28-1B



N28

Test N13-2 against various live loci on M live and test for het

DATE: 9/26/56 REF: N13

	1	2	3	4	5	6	7	8	9	10
	Plate 1.			w2243	w2245	w3112	w3238	w3239	w2244	
			w2243	++	++	++	++	++	++	
			w2245	++	+	+	+	+	6	
			w3164	++	+	0	+	7	0	
10			w3238	++	+	+	+	+	+	
			w3239	++	+	0	+	0	0	
			w3140	++	+	0	+	0	0	
								+ plate 3		
	plate 2			w3133	w3230	w3134	w3157	w3158	w3159	w3089
			N13-2	3	5	5	++	+	1	3
20			w3134	2	7	0	++	+	2	3
			3H3	0	5	3	++	+	0	0
			N23-3	6	10	13	++	+	10	10
			~~N13-2~~							
								plate 5		
	plate 4	w3240	w3148	w3152	w3174	w3156	w3175	w3153	w3237	
30	N13-2	2	2	3	1	3	2	0	6	
	w3134	2	2	2	4	4	0	1	6	
	3H3	0	0	1	0	1	1	2	0	
	N23-3	10	10	10	10	11	10	9	9	
	~~N13-2~~						plate 7			
40	plate 6	w3112	w2243	w2244	w3127	w2245	w3238	w3239	w3147	
	N13-2	8	++	3	5	+	0	++	15	
	w3134	1	++	5	3	+	0	++	2	
	3H3	3	++	0	3	+	0	++	4	
	N23-3	10	++	10	10	+	+	++	10	
50	~~N13-2~~									

+ 24 live

plate 8	W3149	W3215	W3151	W3154
W13-2	8	5	6	1
W3134	1	1	3	3
3H3	1	1	1	3
N23-3	20	8	7	20

DATE: W 3236 P^- Hfr M^- × N6-1 W112 F^- gal $^-$ TL B_1^-

1 2 3 4 5 6 7 8 9 10

~~S gal+M+B_1~~ gal$^+$ (Hfr ? lac$^-$) M^- P^+ ~~lac on S gal+M+B_1.~~

27 A N6-1 in primary. , drop each of W 3236, N6-1 on ~~S~~ S gal + M.+B_1. 29 Replicate on M gal. Spot on B lac.

gal$^+$ lac$^-$ prototrophs cross streaked on Y10 on M lac.

10 One colony N29-6 picked for further tests (Hfr ?).

Streaked on B gal for single colony resolution. N29-2 also picked (Hfr ?).

10/18/56. Test by D(0), D(0) + M.

10/15/56 Both 29-2 & 29-6 are M+ Hfr -1 lac, str$^-$. Purify

20 W29-6 and store as W3221. Purify and retest w29-6.

10/15/56	W3153	Y10	W3089
29-2	+	+	O
29-6	O	+	O
W1946	O	+	+

30

10/18/56. Retest repurified 29-2, 29-6.

	29-2	29-6
W3133	7	O
W3230	+	+
W3089	O	O
W3148	+	+
~~W3189~~		
W3152	+	+
W3174	+	+
W3175	+	+
W3153	+	+

40

Both O.K. = M+ Hfr lac, str$^-$.

50

N. E. Morton
Oct. 4, 1956

Report on lac- study

Preparation of stocks

The plan of this study is to prepare pairs of stocks containing the same lac- allele, the initial member to carry Cavalli's Hfr, M-, and a UV-induced lac-, the other to be a lac- F- prototroph derived from the first by recombination with Y10. For chromosome mapping, each Hfr stock will be modified by selection of V_6^r, a marker closely linked on the left of lac-1. In future, the Hfr stock will also carry P-, one locus for which is reported to lie between lac-1 and V_1^r (Fried, m.s.; her data fit equally well the order P V_6 lac-1). An Hfr P- M- stock W3236 was obtained by UV irradiation of W1895 and is being tested to determine the location of P-. Preliminary tests indicate the order P V_6^r lac or V_6^r lac P.

Pending the development of the P- stock, F- prototrophs and Hfr M- P/ stocks were prepared for the genes lac_1^{y87}, lac_1^{y53}, lac_1^{w112}, lac_2^{45}, lac_4^{w67}, lac_1^{w3229}, lac_1^{w3146}, and for 12 lac- derivatives of W1895 (1940-51). In addition, F- or F/ prototrophs were prepared for lac_3^{w108}, lac_5^{w145}, lac_7^{w133}, and lac^{w3128} (Table 1 and Fig. 1).

In the course of this work, three lac- stocks were isolated which differed in recombination and reversion patterns from the lac- parent. W3159 is a stable isolate from a cross of Y10 with the very highly mutable W1951, and fails to recombine with W1951 and all but one of the apparently single-step lac-1 mutants. W3229 is a spontaneous derivative of W3120 accidentally isolated in serial transfer. It is much more stable than its lac_1^{y87} ancestors and fails to recombine with any of the recognized lac-1 mutants. At present it is the means by which lac-1 is identified, since the lac-1 pseudoalleles have sufficiently high recombination rates to be indistinguishable from unlinked loci in streak tests. W3146 was isolated from a cross of W3129 by W112 in a attempt to introduce lac_1^{w112} into an Hfr stock; it recombines with W112 and all tested lac-1 mutants and is almost certainly

not a derivative of W112, since it remains S^r gal- V_6^r like W3129. (Of the stocks in table 1, the Hfr lac_1^{w112} is the only one not yet prepared.) The origin of the two-step mutants W3229 and W3159 raises questions about the nature and frequency of spontaneous changes in recombination pattern of lac- mutants.

Streak allelism tests

Cross-streaks of Hfr M- lac- and F- lac- prototrophs on M lac plates are convenient tests for allelism, but their interpretation, although clear in most cases, is in others made difficult by too frequent lac+ reversions, especially when they occur in the M- line, and by the relatively low fertility of 3H3, W3164, and W3140. Tests with highly fertile Hfr stocks have been unambiguous.

The lac- stocks tested fall into two groups. The majority fail to recombine with W3229, and are therefore designated lac-1 (Table 2). Of these Y87, Y53, W1950, and W1951 appear to be allelic, but may be separated by their reversion rates, which are in the order Y53 < Y87 < W1950 = W1951 when compared as prototrophs. The latter two stocks are exceptionally revertible and are probably identical, as they were isolated in the same experiment. Similarly, W1948 and W1949 have not been distinguished by recombination and revertibility tests. All other apparently single-step lac-1 mutants recombine with one another. Five lac- genes remain unclassified with respect to locus, since they recombine with lac_1^{w3229}, lac-2, 3,4,5, 7, and lac^{w3128}, as well as with each other. The two recently obtained lac- from W3236 have not been adequately tested. With chromosome mapping tests, some of these unclassified genes will probably be found to be pseudoallelic with known loci.

Intensive allelism tests

Quantitative recombination tests have been deferred until V_6^r P- stocks are available. A few intensive allelism tests were carried out on material at hand, without re-isolation of stocks, so that reversions

in the agar stabs over varying time intervals were confounded with unavoidable reversions in the Penassay broths in which the cultures were grown up and on the M lac plates on which they were tested. Colonies were counted at 24 hrs. to minimize reversions on the plates. Despite the crudeness of these tests, they are of interest in confirming the cross-streak tests and providing a rough measure of reversion rates (Table 3).

W3128 lac- Hist- F+

This stock was received from Borek as a questionable double mutant. Hist + reversions on D(0) remain lac-. Lac- prototrophs were obtained from a cross with W1895. Both hist- and hist + were isolated from lac+ reversions on B lac. All the evidence is consistent with independent origin of hist- and lac-, with hist+ reversions in some lac+ papillae.

Persistent diploids

From H1 lac_1^{y53} colonies were isolated which carried Het, as shown by lacv colonies in the cross with W1940. The lac- parents have been stabbed as N13-2 and the lacv diploids as N13-1.

An attempt was made to test allelism of the lac- segregants of H271, a diploid lac+ which segregates stable and mutable lac-. The original constitution of this stock was lac^{y53}/lac^{w112}, which was lac- in phenotype. Unfortunately, the y53 Hfr tester is of low fertility and the w112 tester has just been synthesized, so a conclusive analysis has not yet been made.

Interaction of lac_1 gal- and lac_1 gal+

E. M. Lederberg reported that cross-streaks of lac_1- gal+ and lac_1- gal- gave a bluish color after 48 hrs. on B lac, but that other lac- loci are negative or give a less intense color. This has been confirmed, the color reaction being much clearer on paper prints than on the agar plate. A gal_1- lac+ tester should be tried. Cells lysed by T6 on B lac agar give a blue reaction, but I was not able to differentiate lac-1 from other loci by this method. In fermentation tests on EMB agar, read at 24 hrs.,

all the lac- prototrophs in this study (with the exception of the mal-1 and gal-2 stocks) behaved as follows:

Locus	mal	mtl	gal	zylose
2 and 3240	slow	slow	≠	≠
3 and 5	0	0	very slow	≠
all others	≠	≠	≠	≠

<u>P1 transduction</u>

Attempts to grow high titer P1 in L broth were unsuccessful on a variety of lp^s stocks. The Swanstrom- Adams confluent lysis plate method is now being tried. As soon as good lysates are made, the transduction system will be explored.

Fig. 1 Pedigree of important stocks

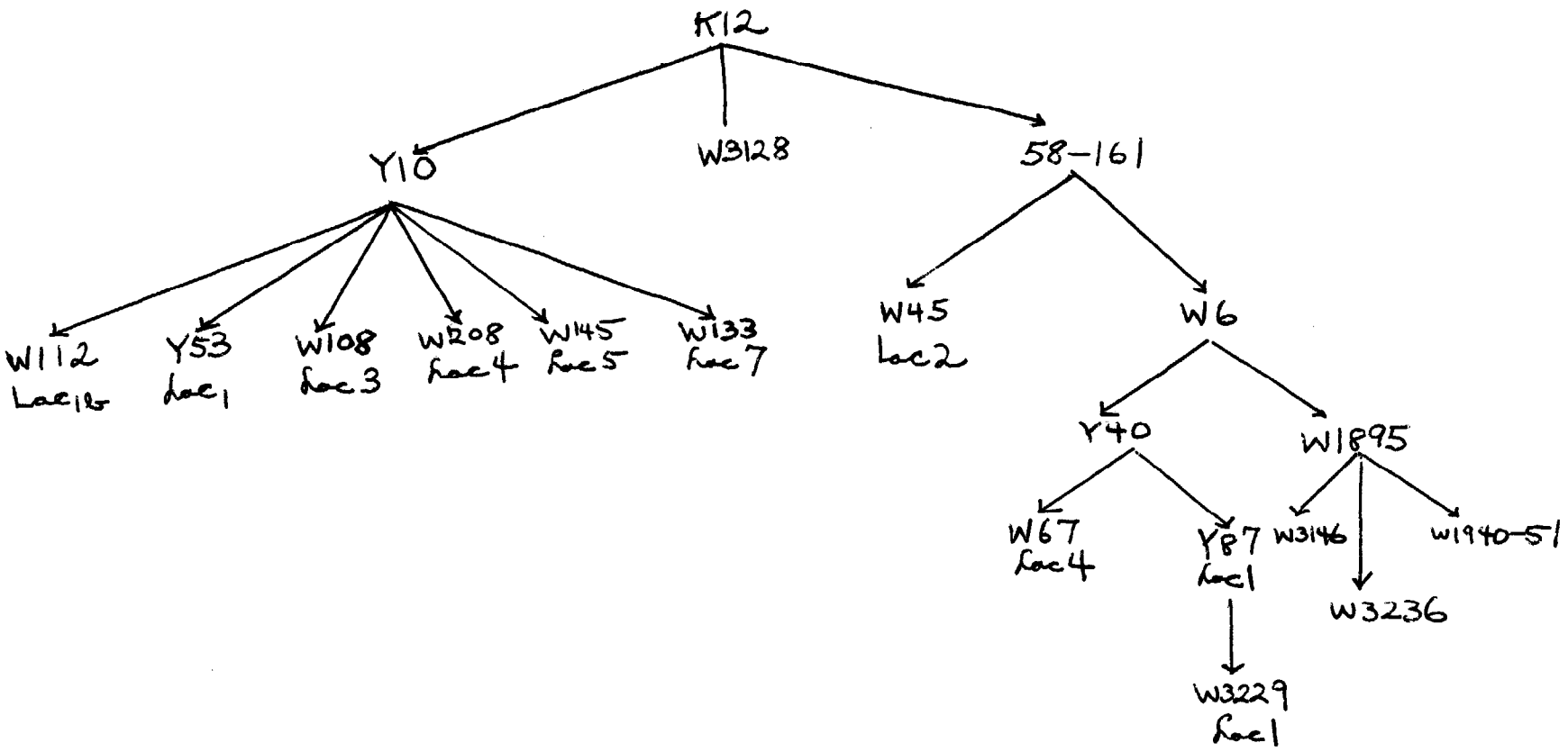


Table 1 Lac Stocks

Source	Locus	Hfr M-	F- prototroph	
y87	1	W3120	W3230	N23
y53	1	3H3 ind. Hfr (JL)	W3134	N2
w112	1	W3221 M+ N6	W3089 mal_1^-	
w1941	1	W1941	W3148	N9
w1945	1	W1945	W3152	"
w1946	1	W1946	W3153	"
w1948	1	W1948	W3174	"
w1949	1	W1949	W3156	"
w1950	1	W1950	W3157	"
w1951	1	W1951	W3158	"
w3146	1	W3146 gal_2^- V_6^r S^r N6	W3175 V_6^r	N6
w3159	1		W3159	N9
w3229	1	W3229	W3133	N1
w45	2	W3164 S^r N5	W3112	
w108	3		W2243	
w67	4	W3140 S^r N4	W2244 F'	
w208	4		W3127	
w145	5		W2245 F'	
w133	7		W3238	N23
w3128			W3239 F'	N7
w1940			W3147	N9
w1942			W3149	"
w1943			W3215	"
w1944			W3151	"
w1947			W3154	"
		Hfr M- P-		
w3237		W3237		
w3240		W3240		

Table 2. Lac_1 recombination pattern

Stocks recombine to give lac+ if the corresponding bars do not overlap.

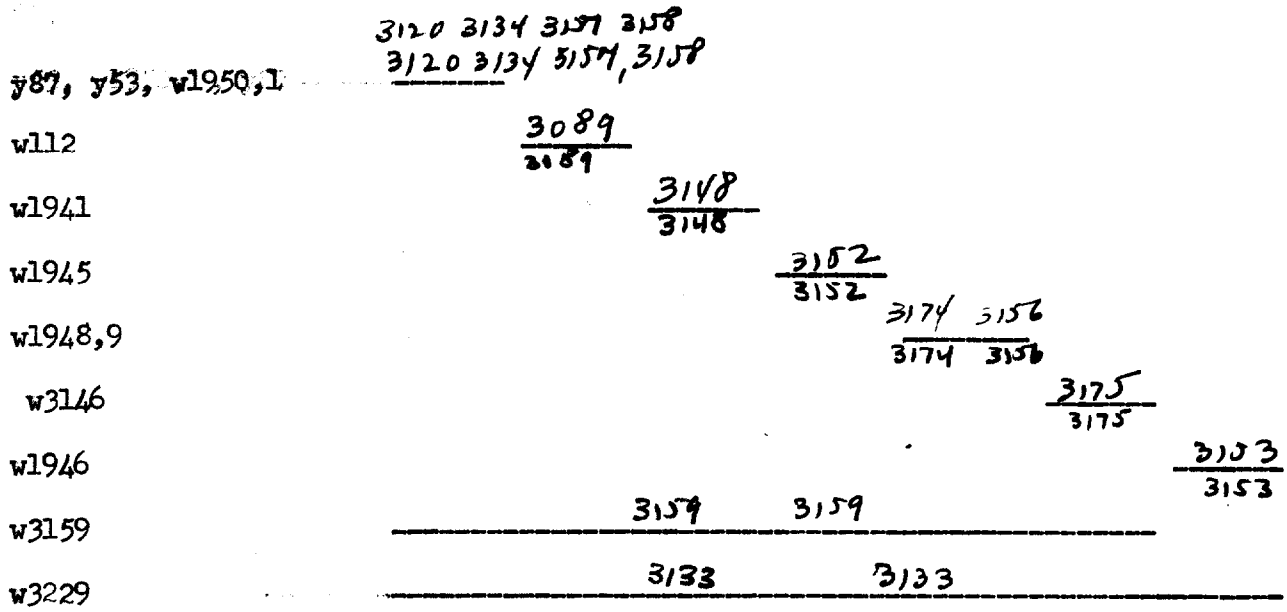


Table 3 Allelism tests

Exper. 1. 0.1 ml. F- and 0.1 ml. Hfr from overnight cultures into penassay. After 4 hrs. plate 0.1 of mix on M lac.

F-	Hfr M- W3229	3H3	W1941
W3133	0	0	0
W3134	22	23	>1000
W3148	1	13	0
W3089	0	—	>1000

Exper. 2. Mix centrifuged, washed with saline, concentrated in saline 1/10. 1.0 ml. of concentrate on M lac.

	W3229	W1941
W3133	3	2
W3089	0	—

Exper. 3. 0.1 ml. F- and 0.1 ml. Hfr in 10 ml. penassay. After 3 hrs. plate 0.1 ml. on M lac.

F-	no Hfr	allelic Hfr		Hfr = W3229
W3133	0	W3229	0	0
W3134	44	3H3	52	50
W3089	0	—	—	0
W3148	0	W1941	0	0
W3152	0	W1945	0	0
W3153	14	W1946	15	11
W3174	0	W1948	0	0
W3156	0	W1949	0	0
W3157	26400	W1950	14200	15400
W3158	29000	W1951	17000	23200
W3159	0	W1951	32	0
W3175	0	W3146	0	0

Table 3 (cont.)

Exper. 4. 0.1 ml. F- and 0.1 ml. Hfr in 10 ml. penassay. After 24 hrs. plate 0.1 ml. on M lac.

F-	no Hfr		allelic Hfr
W3127	32	W3140	153
W3112	0	W3164	0
W3151	0	W1944	0
W3154	1	W1947	1
W3147	3	W1940	2
W3149	1	W1942	0
W3150	1	"	0
W3155	0	"	0

Prep. of high titer P1

DATE: 10/4/56 REF:

Previous attempt to prep. high titer stock in L broth failed; all stocks gave < 10^8/ml. (N20). A subsequent attempt with confluent lysis plates gave incomplete lysis, yields less than Lennox P1 (N25). Plaques on L agar are pinpoint size only against W1485.

11:30 AM. W1895, W1366 into L broth. Rotate.

3PM. Pour plate 1 drop Lennox P1.

8PM. Complete lysis (too much phage). add L ø broth.

10/5/56. Decant broth, chloroform, spin down, transfer to frig. W1895 prep is contaminated (yeasty smell, milky broth). Discard. W1366 is all right. Much more lysis with these Lp+ stocks than with Lp-.

10/8/56. Streaks on Lø against W3236.

10/9/56 P1(1366) and P1(1895) give good lysis by comparison with P1 from Lennox. (untitrated into λ (?). P1(W1485) very sick. Try D(M) + Ca^{++} on broth + W3089 and W3089 on Rotator.

10/12/56. Lysis from D(M) broth not as clear as from L ø layer plates. P1(W1366) agar layer plate produces as much lysis as Lennox phage. B(O) + Ca^{++} plates are not as clear as Lø.

W3236(P1) prepared on Lø plate. resistant to P1 by comparison with W3236.

11/6/56. also resistant to T1 (see S. Luria).

N31

loc v ? from N13-2 X other loc

DATE: 10/4/56 REF:

	1	2	3	4	5	6	7	8	9	10
W	3112	W2244	W3127		no loc v isolated.					
	31-1	2	3							
10										
20										
30										
40										
50										

Linkage test of W3236 XW945

DATE: 10/8/56. REF:

W3236 = M⁻ Hfr P⁻

W945 = gal₂⁻ lac₁⁻ (T<B₁)⁻

10 A.M. mixture in primary. Plate $X10^{-2}$ on D(0) + prol.

10/9/56. Streak on ¼ for single colony isolation.

Test on 8 gal, 8 lac, and 5 lac M. 60× colonies.

all gal —.

	lac⁻ Prol+	lac⁻ Prol−	lac+ Prol+	lac+ Prol−
plate 1	17	1	IIII	IIII
plate 2	23	11	III	##### ###
Total	40	3	6	14
%	63.5	4.8	9.5	22.2

gal lac P

lac+/lac⁻ 20/43

prol+/+ 17/43

$\frac{20+2(3)}{60}$

lac+ prol+ 6

prol⁻ lac+ 3

gal Prol lac

$\frac{17+3}{60}$ $\frac{3+9}{60}$

Indecisive with respect to proline location.

See next page.

N32A

DATE: REF:

H1

gal I Prod II V_6 III lac IV V_1

doubles I 22.2 + 4.8 = ~~27.0~~

II + III 9.5 + 4.8 = 14.3

I, II, III 22.2 + 9.5 + 2(4.8) = 41.3

gal Prod V_6 lac

20.6 14.2

27.0 14.3 41.3

II. $P^- V_6^+ + P^+ V^S$ = (2.8) + 5.7 + 7.8 + 4.3 = 20.6

III $V_6^+ lac^+ + V_6^S lac^-$ = 5.7 + (2.8) + 4.3 + 1.4 = 14.2

34.8

H2

gal V_6 lac Prod. V_1

I II III IV

III 9.5 + 4.8 = 14.3

I + II lac^+ 9.5 + 22.2 = 31.7

I + II + III 2(9.5) + 22.2 + 4.8 = 46.0

II $V_6^- lac^+ + V_6^S lac^-$ = 5.7 + 2.8 + 4.3 + 1.4 = 14.2

III $lac^- Prod^- + lac^+ Prod^+$ = 5.7 + 1.4 + 5.7 + 7.8 = 20.6

II + III 34.8

34.8

gal V_6 lac Prod

14.2 20.6

31.7 14.3

46.0

Under H1 doubles 3 singles 60

under H2 10 13

6 5

10 13

N33

DATE: 10/9/56 UV induced loc in W3236. REF:

1	2	3	4	5	6	7	8	9	10

~40 single colonies of W3236 picked from B loc into penicillin. 1 ml from 0 overnight cultures per plate B loc. UV 10 sec!

10/11.

	W3266	N33-1	loc-
10	W3267	N33-2	loc-
	~~W3268~~	~~N33-3~~	~~[redacted] dead~~

20

30

40

50

W344

DATE: Transductions with P1/lac REF:

10/10/56 .1 ml W3236 on S lac + M + 0.1 ml. P1 (W1366).
control: 0.1 ml W3236, 0.1 ml P1 (W1366).
10/12/56

W3236 blank
P1 (W1366) blank
W3236 + P1 (W1366) ca. 100 protoprophs gal lac. ?

Streak out 8 colonies and test remainder on

M lac + Y10		+ +	lac +
S lac			
S lac + with		+ +	lac +
S lac + M + T6		+ +	lac +

The colonies streaked out on lac +.

10/16/56. Repeat on S lac. add T6 after 2 hrs.

0.1 ml W3236
0.1 ml P1 (W1366)
0.1 ml W3236 + 0.1 ml P1 (W1366)
Repeat on S lac + M

1	.05 ml W3236		a 2 part test on S lac + M gives 10 lac + from W3236 + P1 (W1366).
2	.05 ml P1 (W1366)		
3	.05 ml P1 (W1366) + W3236	+ (ca. 200 colonies)	

10/17/56. Colonies from 34-3 streaked on S lac. Purified all lac +.
colonies from 10/12/56 streaked on Y10 on M lac for H fr test. all H fr.

1	5
2	6
3	7
4	8

P1 (W3089) from DM + Ca++ broth on W3100 on M lac +.
P1 (1366) from agar lysate on W3127 on M lac <

1-1	.05 ml 3127	2-1	.05 ml 3127
1-2 (W3469)	P1 .5 ml.	2-2 (W1366)	P1 .05 ml < O.K.
1-3	.05 ml 3127 + P1 (.5 ml)	2-3	.05 ml W3127 + P1 (W866)

Use W3140 many reversions contaminated w. bacteria

many reversions

see N37. (over)

10/18/56.

N 35

Tests of Y70 = lac⁻ (from Y53) TC^S, F⁻

DATE: REF:

10/14/56 1 cracked tube from hyphil into Penassay (2 left, O.K.)

10/15/56 No growth. 1 good tube from hyphil into Penassay (1 left).

10/16/56. hyphil o.k. Tests streaked against lac 1 H for 2 spots on Mac allelic lac F⁻ control and Y10 control

		+Y70		+allelic F⁻	Y10
10	W3229	0	3133	0	++
	W3120	0	3230	20	+
	29-2	3	3089	0	++
	29-6	4	3089	0	++
	1941	3	3148	0	++
20	1945	5	3152	0	++
	1948	2	3174	0	++
	3146	6	3175	0	++
	1946	1	3153	10	++
	W3236	15	Y70	10	++

10/20 Repeat using new tester of H for and ~~Y53~~ Y53 control.

10/21.

	Y53	Y70	Y10	
W3229	0	0	+++	
W3120	0	0	+++	
W3221	+	+	+++	35-1
40 W1941	++	+	+++	
W1945	++	+	+++	
W1948	+	+	++	
W3146	+	+	++	35-2
W1946	+	+	++	
50 W3236	++	++	++	
W3140	+	+	+++	

∴ Recombination pattern of Y70 not different from Y53

N36

W3120, W1950 UV
look for lac stable in B lac.

DATE: 10/16/56 REF:

10 sec. UV (1 drop). 12 plates of W3120 (1), 12 of W1950 (2), 10 of W3236 (3)

10/18 lac-stable colonies of W1950, W3120 into Penassay for allelism test. Two doubtful lac streaked on B lac.

10/19. One lac from W3236 = N36-1 (W3268) streaked on B gal for stab.

Test of lac stable W3120, W1950 controls

		36-1A	1-B	1-C	36-2A	36-2B
36-1	W3089	O	+	O	O	O
2	W3148	O	+	O	O	O
3	W3152	O	+	O	O	O
4	W3156	O	+	O	O	O
5	W3175	O	+	O	O	O
6	W3153	O	+	O	O	O
7	W3133	O	+	O	O	O

10/21. Four colonies (2 from W3120) 2 from W1950) clean on all indicators. One other clean on W3175 only. Overnight broths into M lac.

	1A	1B	1C	2A	2B
Y10	++	++	++	++	++
W3133	O	+	O	O	O
W3175	O	++	O	O	O
W3153	O	++	O	O	O

Streaks of 1A - 2B onto fig.

10/23 Discard 1B. Stab others for single colonies on B-O.

~~1B 1A 2A 2B~~.

1A = 3269
1C = 3270
2A = 3271
2B = 3272

N37

W1366 loc$_1^{wr2}$ $V_6^-V_1^-$ TCB$_1^-$ xW3236 M$^-$Hf$^-$P$^-$

DATE: 10/19/56 REF: N34

	1	2	3	4	5	6	7	8	9	10

10/18/56. Mutation, phage sensitivity and resistance of single colonies o/k.
10/19 1 drop of each on DO+ prob. Spread from this to 2 other plates.
10/20 in frig. 10/21 streak out single colonies on B-O.
10/22 Pick independent on DO+ prob. 10/23 in frig.
10/25. Replicate on DO, Blue. Streak on B-O+T1, B-O+T6.

20

30

40

50

N37A

Tested 200 discrete colonies from N37. Clear replication of long streaks on B-O+T1, T6.

DATE: 10/29 REF:

plate no.	a V6	b Lac	c Prol	d V4	e	5	V6	Lac	Prol	V4	10
11											
13											

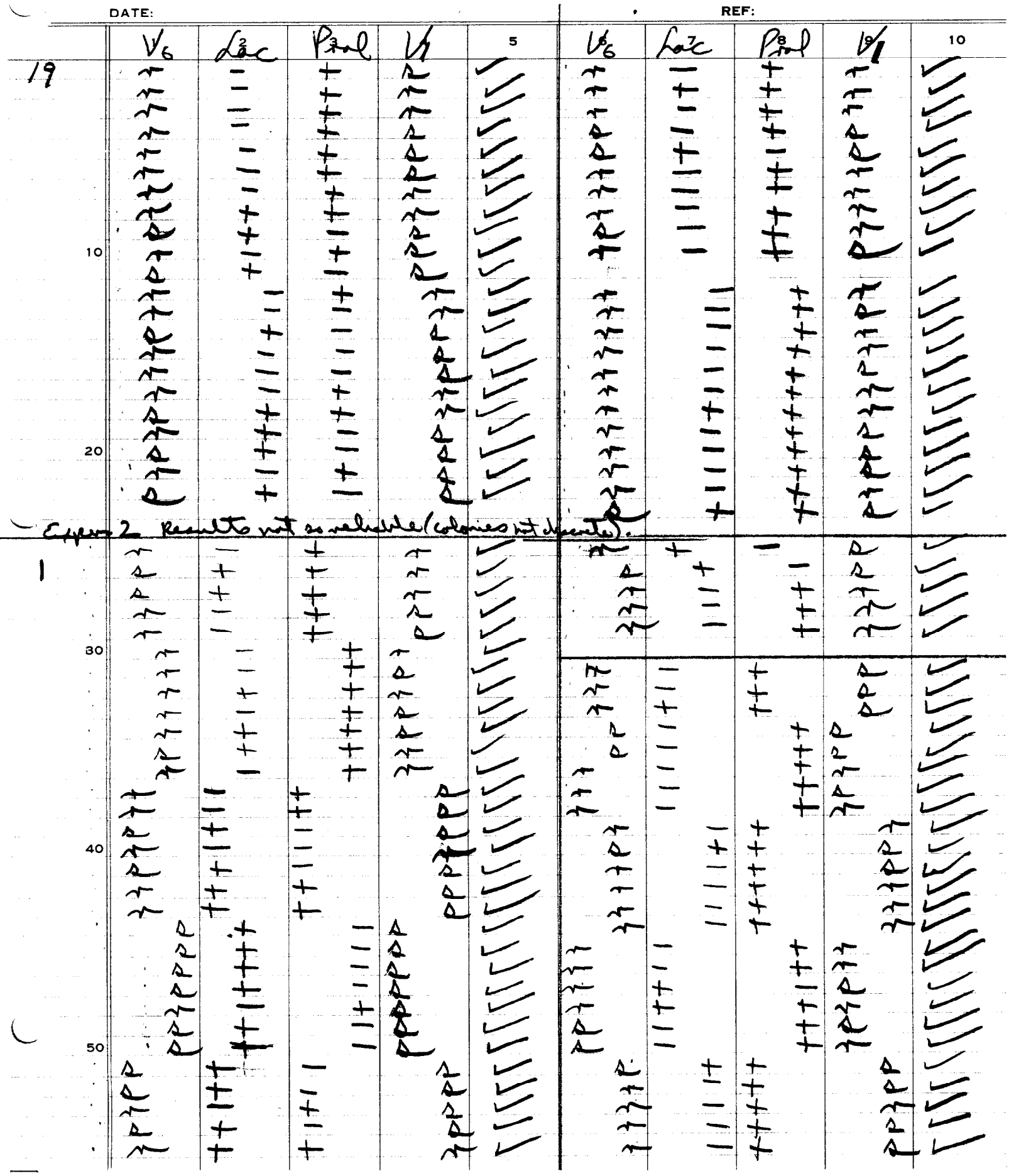
N37B

DATE: REF:

	V6	Lac	Pnl 3	V4	5	V6	Lac 7	Pnl 8	V1	10
15										
10										
20										
17										
30										
40										
50										

N37C

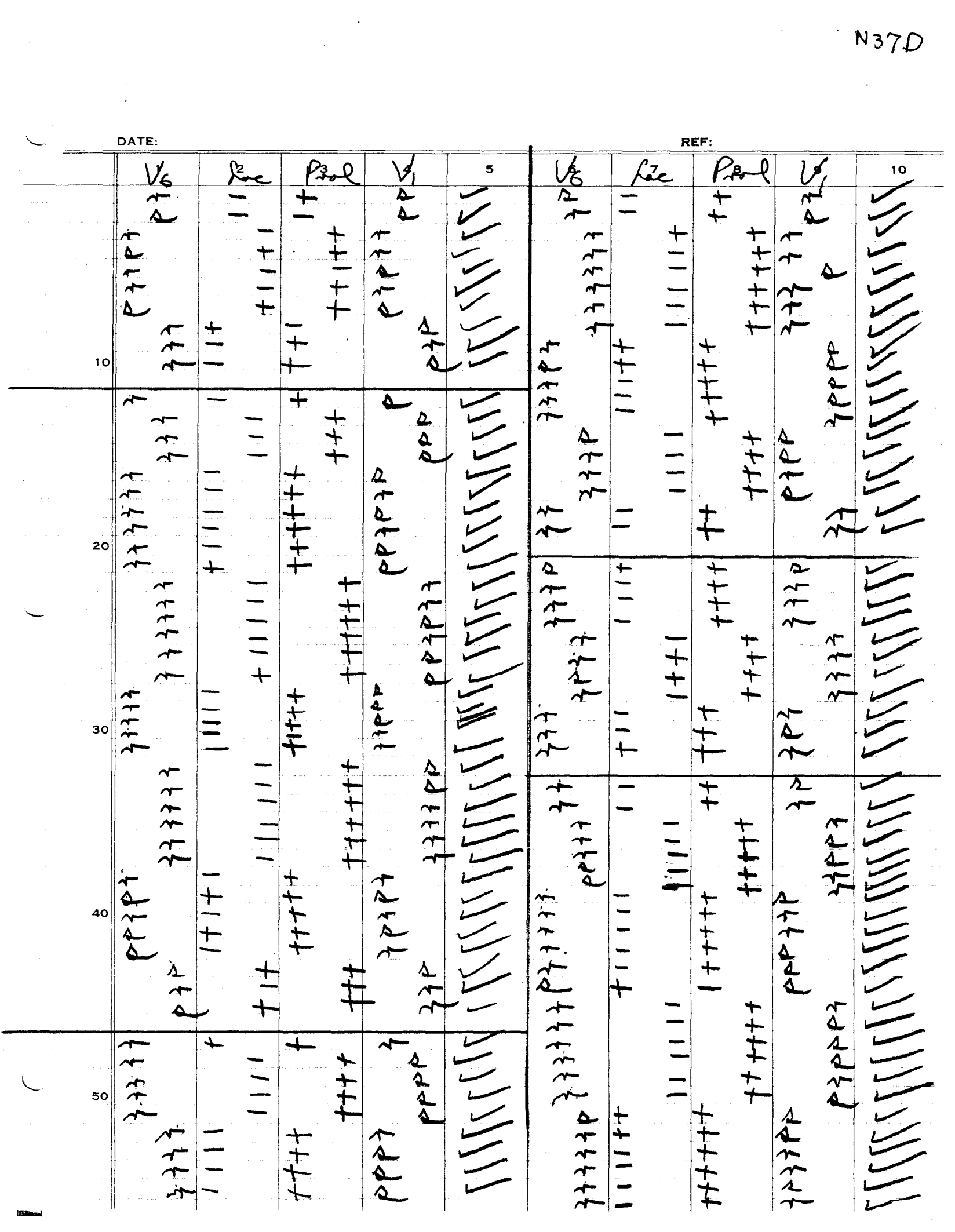
DATE: REF:



N37D

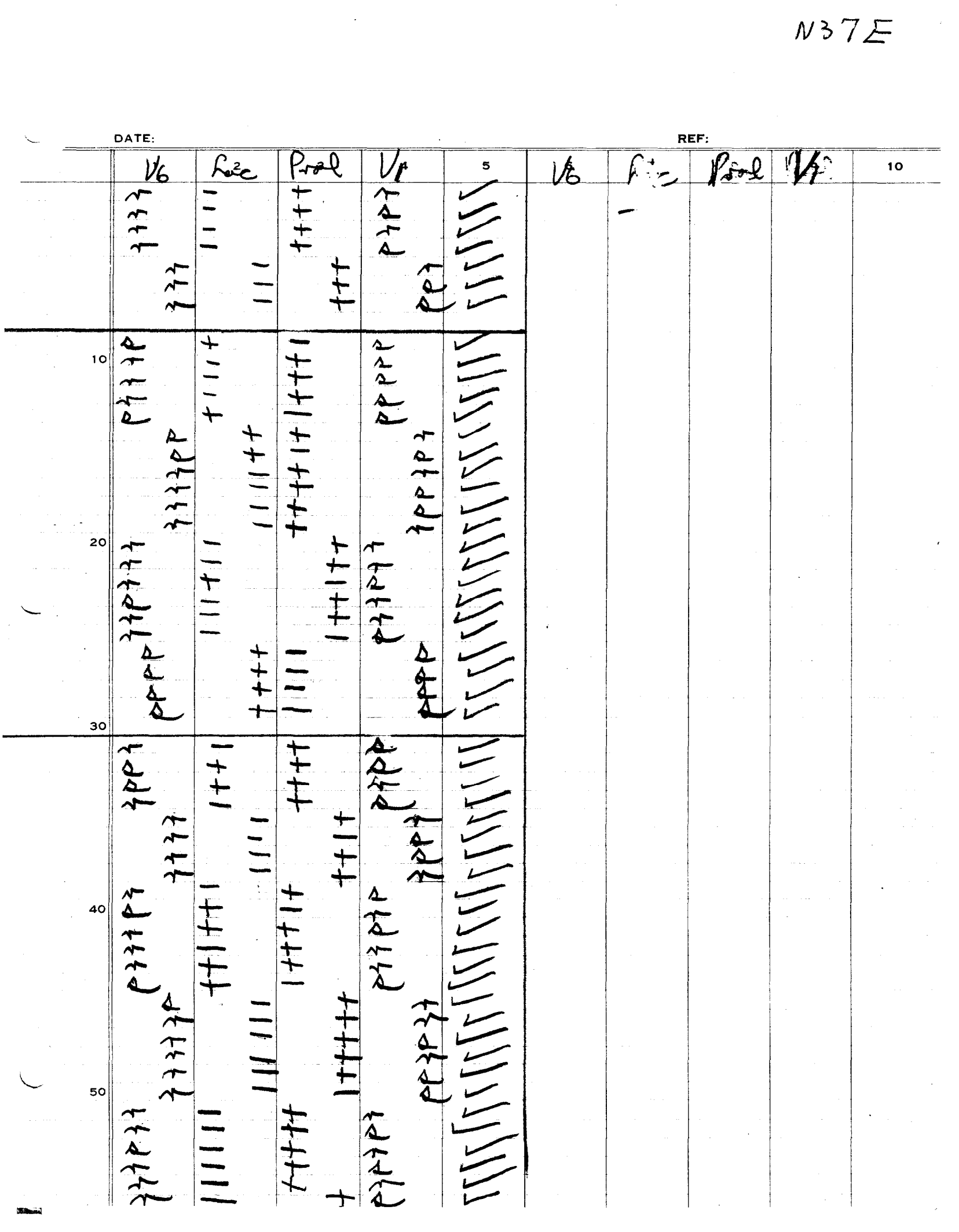
DATE:

REF:



N37E

DATE: REF:



Summary of experiments 21, 32, and 37

Mapping Δ, V_6, lac(1), Pro(1), V_1, (TL).

DATE: 11/2/56 REF:

all experiments were carried out by plating the two parents on D(0)+ proline. Except in experiment 37A, there was contact between some of the colonies. Under the denser growth of the other experiments there was apparently some selection for P+.

Exper.	P−	P+	Total	%	Lac+	V_6^S	V_1^S	%
21	28	113	141	19.9	35	31	~~—~~	
32	17	46	63	27.0	20	—	—	
37A	84	134	218	38.5	79	76	136	
37B	32	192	224	14.3	61	52	128	

assuming ~~no~~ independent selection for other factors [an obvious oversimplification because of linkage to Δ, Pro(1), (TL)], an unbiased estimate of the recombination fraction between two markers is gotten from the determinant r of their 2 × 2 table, viz.

$$r = \begin{vmatrix} a & b \\ c & d \end{vmatrix}$$

, where a and d are the recombinant classes and the recombination fraction is estimated as $\hat{\theta} = \frac{\sqrt{r}}{1+\sqrt{r}}$,

$$\sigma_{\hat{\theta}}^2 = \frac{\theta^2(1-\theta)^2}{4}\left\{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}\right\}$$

$$I_{\hat{\theta}} = 1/\sigma_{\hat{\theta}}^2$$

Summary experiments 21, 32, & 37

37A weighted X2

DATE: REF:

Region	Exper.	a_2	b_2	c_4	d_6	$\frac{ad}{bc}$	$\sqrt{\frac{ad}{bc}}$	$\hat{\theta}^8$	$I\hat{\theta}$ product	$\hat{\theta}^{10}$ terminal
$\Delta - V_6$	21	31/141						~~.220~~		
	37A	76/218						.349 ✓		.349
	37B	52/224						~~.232~~		
$\Delta - loc_1$	21	35/141						~~.248~~		
10	32	20/63						~~.317~~		
	37A	79/218						.362 ✓		.362
	37B	61/224						~~.272~~		
$\Delta - P$	21	28/141						~~.199~~		
	32	17/63						~~.270~~		
20	37A	84/218						.385 ✓		.385
	37B	32/224						~~.143~~		
$\Delta - V_1$	37A	136/218						.624		.608
	37B	128/224						.571		
$V_6 - loc$	21		20/141 = .142			.042591	.20637	.171		
30	37A			33/218 = .151		.03569	.18892	.159	.165	.148
	37B			33/224 = .147		.04172	.2042	.170		
$V_6 - P$	21		29/141 = .206			.14296	.3781	.274		
	37A			57/218 = .239		.10913	.3303	.248	.245	.213
	37B			38/224 = .170		.06962	.2639	.209		
$V_6 - V_1$ 40	37A			88/218 = .404		.24573	.4957	.331	.360	.430
	37B			108/224 = .482		.5111	.7149	.417		
$loc - P$	21		29/141 = .206			.1226	.3501	.259		
	32		7/63 = .143			.03214	.1793	.152	.209	.193
	37A		47/218 = .216			.08331	.2886	.224		
50	37B		41/224 = .183			.05145	.2268	.185		
$loc - V_1$	37A		81/218 = .372			.31216	.5587	.358	.369	.398
	37B		101/224 = .451			.41082	.6409	.391		

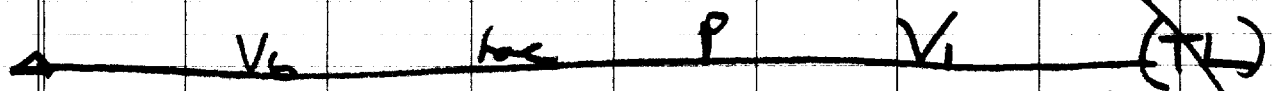
Summary of Experiments 21, 32, and 37

DATE: REF:

Region	Exper	a	b	c	d	$\frac{ad}{bc}$	$\sqrt{\frac{ad}{bc}}$	ϕ	I_ϕ	10
$P - V_1$	37A		$\frac{68}{218} = .312$			.08535	.29215	.226	.220	.357
	37B		$\frac{102}{224} = .446$			.0695	.26363	.209		
$V_6 - (T2)$	37A	$\frac{82}{218} = .376$								.376
	37B	$\frac{96}{224} = .429$								.394

Binomial

Estimates assuming $\theta = w - \frac{1}{2}w^2$



.349	.148	.193	.357	.394		$\Sigma = 1.441$
.362					.497 − .124 = .373	
.385					.690 − .238 = .452	
.608					1.047 − .548 = .499	
	.148					
	.213				.341 − .058 = .283	
	.430				.698 − .244 = .454	
	.651				1.092 − .596 = .496	
		.193				
		.398			.550 − .151 = .399	
		.638			.944 − .446 = .498	
			.357			
			.615		.757 − .282 = .475	
				.394		

$w - \frac{1}{2}w^2$

N38

DATE: 10/19/56 REF:

1	2	3	4	5	6	7	8	9	10

+meth

M lac ± W3140 ± P1(W1366) 1 drop ea.

38-1	M lac + W3140	0	38-4	~~P1~~ P1(W3089)	0
38-2	+ P1(W1366)	0	38-5	W3140 + W3089	0
38-3	+ W3140 + P1(W1366)	ca. 300	~~38-6~~		

10/21 Streak from 38-3 on B lac.

10/22. Several possible heterogenotes streaked on B lac.

10/23 ~~Opaque~~ Second streak (Some lac⁻ in first, probably parental).

10/24 No lac⁻ in second streak; some bull's eye colonies ◉.

10/25 Streak from periphery of bull's eye. 4/6 all lac⁺.

11/6/56. M lac + M tet + W3140. 11/7/56 Streak on B lac.

Streaked on B-O + T6 and B lac + T6. 1/73 colonies V_6^+ (on B-O).

This streaked on B-lac + T6, with several possible lac⁻ sectors from V_6^+.

11/15/56. The V_6^+ colony lac⁺. No heterogenotes found. lac⁺ and lac⁻ colonies streaked on B lac⁺ T6.

11/16/56. all V_6^S.

N39

DATE: 10/19/56 W3236 UV on Blue REF:

2 drops, 12 sec., 17 plates. 10/21/56 No lac.

Repeat, 15 plates. 11/28/56.

11/29/56. Three lac. Streaked on B-O for stab.

39-1 mal- glucose-?

39-2

39-3

N40

allelism Tests

DATE: 10/21/56 REF:

O — M	loc	2	3	4	5	6	7	8	9	10
	w3237	w3240	w3266	w3267	w3268	w3236				
w3133	++	O	++	++	++	++				
w3112	+	O	+	++	++	++			40-1	
Y10	++	O(3)	++	++	++	++				
w2243	—	—	—	—	—	—	—	++		
~~w2244~~ w3127	++	O	++	++	++	++				
w2245	—	—	—	—	—	—	++		40-2	
w3238	++	O	++	++	++	++				
w3239	++	O	++	++	++	++				
w3147	++	O	++	++	//	//				
w3149	++	O	++	++	//	//			40-3	
w3215	++	O	++	++	//	//				
w3151	++	O	++	//	//	//				
w3154	++	O	++	//	//	//				

10/22. w2243 and w2245 are largely reverted to loc+.

galactosidase tests

N#8

DATE: 11/2/56 REF:

	1	2	3	4	5	6	7	8	9	10

Hexaploid made YZ + ½% lac + ½% glycerol.

YZ + lactose broth. ^ Lac+ sticks streaked on B-O.

11/4/56 Single colony sticks from B-O into YZ + lac. Rotate overnight.

A5 Spin down. ~~Discard~~ Discard supernate. ^ add 1 ml H_2O + 1-2 drops Streak on Blue.

benzene to ~~cap~~ pellet, shake well. 0.1 ml of this mix + 2 ml. H_2O

10 + 0.1 ml. ONPG (30 mg/20 ml.)

Read at (0 min.

		ONPG	Blue			ONPG	Blue	
lac+	W3236	++	+		W1942	O	✓	
1	W1949	+	✓		W3240	O	✓	
	W3266	O	✓		W1940	O	✓	
1 20	W3159	+	✓	1	W1950	+	trailer	
*	W3270	O	✓	lac ctt.	W3239	+	✓	also + from glucose
1	W3221	+	✓		W1943	O (±)	✓	W3244 slow + from glucose
*	W3269	O	✓		W3237	O	✓	streaks from Blue on lac.
1	3H3	+	✓	1	Y70	✦	✓	
1 30	W1941	+	✓	1	W1951	+	✓	
	W1947	O	✓		W3272	O	✓	
1	W1948	+	✓		W3164	O (±)	✓	
*	W3267	+	all +	slow + no good /	W1945	+	✓	
1	W3120	+	✓	slow + usually				
* 40	W3268	+	all +	slow + 0.1(C)	± = very faint yellow tinge.			
1	W3146	+	✓		Of 6 two-step lac-1 mutants, only one (W3159) is ONPG+. Also, Y70 is ONPG+.			
*	W3229	O	✓		2) all recognized ^ single-step lac-1 mutants are ONPG+.			
*	W3271	O	✓		3) Other ONPG+ are W3267 & W3268.			
rec-?	W3238	O	✓		~~Also two of five ONPG+.~~			
1 50	W1946	+	✓		Revertants W2243, W2245 W3268, W3267, W3238,			
	W1944	O (±)	✓		W3140, and Davis cat lac+			

11/23/56. 0.1% W3159, W3267, W3268 rechecked; all ONPG+.

N 42

allelism tests of w3269-72, w3240 against w3127

DATE: 11/3/56 REF:

1	2	3	4	5	6	7	8	9	10
On M lac			w3133	w3127	Y10				
	w3269		O	O	++				
	w3270		O	O	++				
	w3271		O	O	++				
	w3272		O	++	++				
	w3240		O	O	+ (ca. 50).